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# THE RELATIONSHIPS OF THE VIREOS (VIREONINAE) AS INDICATED BY DNA-DNA HYBRIDIZATION

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The New World passerine subfamily Vireoninae contains 43 species in four genera. *Vireo*, the largest genus, is primarily North American but several of its 25 species occur on Caribbean islands, one species has a subspecies in Bermuda, and two species occur as far south as Argentina. The 13 species of Neotropical greenlets (*Hylophilus*) are primarily South American; three occur in Central America of which one reaches southern Mexico. *Vireo* and *Hylophilus* are the "typical" vireos, being relatively small passerines with green, yellow and gray plumage colors. The other two genera, *Vireolanius* and *Cyclarhis*, are larger, brighter in color, and with heavy, shrike-like bills. The three species of shrike-vireos (*Vireolanius*) occur from southern Mexico to Bolivia, and the two species of pepershrikes (*Cyclarhis*) range from southern Mexico to Uruguay (Thomson 1964, Blake 1968).

Opinions about the relationships of the vireos to other oscine passerines (Passeres) have focused primarily upon two groups, namely: the shrikes (Laniidae), and the New World nine-primaried oscines, particularly the wood warblers (Parulini of Sibley 1970; Sibley and Ahlquist 1982b).

The vireos have been thought to be related to the shrikes because they have a shrike-like bill with a hooked tip and a subterminal notch in the maxillary tomium (e.g., Coues 1892). The principal basis for placing the vireos near the nine-primaried oscines has been the size and coloration of the typical vireos and their tendency toward the reduction of the outer (10th) primary (e.g., Mayr and Amadon 1951:27).

In this paper, we present data from quantitative comparisons of the single-copy DNA sequences showing that the vireos, greenlets, peppershrikes and shrike-vireos are closely related to one another, and that they are not closely related to the New World nine-primaried oscines, but are members of a large "corvine" assemblage that includes the corvids (Corvidae), shrikes (Laniidae), drongos (*Dicrurus*), monarchs (*Monarcha*), cuckoo-shrikes (Campephagidae) and several other groups. The following review of the taxonomic history of the vireos demonstrates the difficulties and controversies involved in the discovery and interpretation of the traditional characters that have been used as the basis for opinions about the relationships among birds in general, and the vireos in particular.

## TAXONOMIC HISTORY OF THE VIREOS

Baird (1858) first included the Vireoninae in the Laniidae, because of the "same abrupt and lengthened hook" at the tip of the bill, but by 1864 he had placed the vireos in a separate family, Vireonidae. Sclater and Salvin (1873) agreed with Baird (1864) but Sundevall (1872) placed *Hylophilus* and *Cyclarhis* (plus *Dulus*) in the "Fam. Hylophilinae," and *Vireo* in the "Fam. Vireoninae."

To Parker (1878:282–284) the skull of *Cyclarhis* was so similar to that of "*Suthora bulomachus*" (=*Paradoxornis webbianus*, Webb's Parrotbill) of China that he despaired of discovering "the laws of the geographical distribution of birds." Parker considered *Paradoxornis* to be a parid and dubbed *Cyclarhis* "this large archaic Tit," but he left *Cyclarhis* in the Vireonidae. *Paradoxornis* was placed in the Panurinae of the Timaliidae by Deignan (1964).

Gadow (1883) included the vireos as a subfamily of his Laniidae primarily because of the bill characters. Other subfamilies in Gadow's Laniidae were the Gymnorhininae, which included the Australian magpies and butcherbirds (Cracticidae) and the Bornean Bristlehead (*Pityriasis gymnocephala*). The vanga shrikes (Vangidae) of Madagascar, and some of the African and southeast Asian shrikes, were placed in the Malaconotinae. Gadow's (1883) Pachycephalinae included the Australo-Papuan genera *Falcunculus*, *Oreoica*, *Eopsaltria*, *Pachycare* and *Pachycephala*. These may seem like strange companions for the New World vireos, but, as will become apparent, Gadow was closer to the mark than the many later taxonomists who allied the vireos to the wood warblers. (See Sibley and Ahlquist 1982b.)

Coues (1892:329) considered the vireos to be "small dentirostral *Oscines*, related to the Shrikes, with hooked bill, 10 primaries and extensively coherent toes." Coues placed the vireos in their own family, next to the Laniidae, and he commented (1892:331): "But that the important character of number of primaries—one marking whole families . . . should here subside to specific value only, seemed suspicious; and the fact is that all the species really have 10, only that, in some instances, the 1st primary is rudimentary and displaced, lying concealed outside the base of the second quill."

Ridgway (1904:233) placed the Vireonidae next to the Laniidae but expressed "great doubt whether the . . . groups comprising Dr. Gadow's Laniidae . . . can, any of them, be properly included in the same family . . ." with the true shrikes.

Pycraft (1907:375) noted that the Vireonidae were thought by some to be related to the Sylviidae, by others to the Laniidae but, in his opinion

(which was based upon skeletal comparisons) only *Cyclarhis* is a shrike, *Vireolanius* is related to the Artamidae, and *Vireo* to the Muscicapidae.

Naumburg (1925) observed that "the bill of *Cyclarhis* . . . is very similar to that of *Falcunculus* [of the Pachycephalidae of the Australian region] which it also resembles so much in other characters as usually to induce authors to bring the two together either as adjacent genera or as belonging to closely allied sub-families."

Wetmore (1930) took "the work of Hans Gadow" as his starting point and incorporated such changes "as seem justified from personal research or from the investigations of others." This led Wetmore to place the Cyclarhidae and Vireolaniidae near the shrikes, but to dissociate the typical vireos from them and to place his Vireonidae between the nine-primaried Old World white-eyes (Zosteropidae) and the Neotropical nine-primaried honeycreepers (Coerebidae), and close to the other New World nine-primaried oscines, including the wood warblers. This arrangement seems to have been based, in part, upon Pycraft's (1907) claim that only *Cyclarhis* is a shrike and that *Vireolanius* is related to *Artamus*. This is indicated by Wetmore's (1951:11) opposition to the opinion of Zimmer (1942:10), who had expressed his belief that *Cyclarhis* and *Vireolanius* should not "be given family distinction from the Vireonidae. They are heavy-billed, heavy-footed vireos, with a vireonine pattern of coloration . . . and vireonine habits . . . . I should be loath to place a subfamily Vireolaniinae in the family Artamidae" as suggested by Pycraft (1907).

In reply, Wetmore (1951:11, 1960:20) argued that "while Zimmer [1942] believes that the family Vireolaniidae should be included in the Vireonidae, separate family rank in my opinion is definitely justified. In addition to the characters assigned by Pycraft for the shrike-vireos, I have found recently that in the pterylosis the dorsal tract . . . is forked, the arms . . . broad . . . separated from the . . . line . . . onto the caudal area. This is . . . different from the usual rhomboid found in the vireos, and may indicate that the family eventually should be removed from the vicinity of the Vireonidae." Wetmore (1951) also referred to the skeletal characters "outlined by Pycraft [1907]" to support his family distinction for the Cyclarhidae. Wetmore used the same arrangement of three separate families in the last edition (1960) of his classification.

Wetmore's (1951, 1960) association of the typical vireos with the New World nine-primaried assemblage apparently was dictated by his belief in their distinctness from the shrike-vireos and peppershrikes, coupled with the tendency for the reduction of the 10th primary in *Vireo*.

Hellmayr (1935) used a relatively neutral sequence of families: Vireonidae, Vireolaniidae, Cyclarhidae, Laniidae, Sturnidae, Coerebidae and Compsothlypidae (=Parulidae). Mayr and Amadon (1951:21) viewed the

shrike-like bill of the vireonids as due to convergence and placed the Vireonidae (including *Vireolanius* and *Cyclarhis*) in their "Vireos, Finches, and Allies" which included the New World nine-primaried groups.

From his study of jaw muscles and other anatomical characters Beecher (1953) developed an intriguing combination of the shrike and wood warbler theories of the relationships of the vireos. He wrote (1953:273) that "judging from anatomical and other characters, the . . . vireos . . . are apparently descendants of the Old World insect-eaters that were cut off when the northern exchange corridor submerged or became too cold. Subsequently, the vireos gave rise to the entire nine-primaried American assemblage. They appear directly ancestral to the . . . tanagers and . . . warblers . . . the Oscines existed only as insect-eaters when the Vireonidae became isolated in the New World, and . . . this happened *before* the origin of the flowering plants or about the same time." Beecher (1953:294) included the vireos with the monarchs, whistlers (*Pachycephala*) and drongos in the family Monarchidae, characterized by "a winged ectethmoid, [with] a large single foramen, a fused lacrymal . . . a prominent postorbital process . . . [and] specialized bills . . . ." Beecher (1953:324) placed his Monarchidae next to "The American Nine-Primaried Assemblage" and concluded that "it seems particularly clear that the American nine-primaried families arose from the vireos (Vireoninae), a subfamily of the Monarchidae" (Beecher 1953:305).

Beecher's (1953:324) phylogenetic tree splits the oscines into two "superfamilies," Sylvioidea and Timalioidea. We have found that the prototypes of these two groups, the sylviine warblers and timaliine babblers, are actually ecotypes of a monophyletic cluster, so closely related that we have felt obliged to include both groups in the Sylviidae (Sibley and Ahlquist 1982c).

Thus, although much of Beecher's (1953) oscine phylogeny is untenable, he did find evidence of relationship between the vireos and the monarchines, with which we agree. His attempt to derive the American nine-primaried assemblage from the vireos may have been conditioned by Wetmore's influence.

Tordoff (1954a:7), "largely on the basis of studies by other authors," included the Vireonidae as the first family in the sequence of "Vireos, finches, and allies" (1954a:32). Tordoff (1954b) criticized Beecher (1953) for thinking "that the Vireonidae . . . have existed *as vireos* in the New World since some time in the Cretaceous" and identified the "Fringillidae" (by which he meant the cardinals, the tanagers, and the genus *Fringilla*) to be "the central stock" of the New World nine-primaried oscines, while the vireos "are shown to be derivable from primitive finches . . . ." (1954b:283).

Stallcup (1954) reported that certain features of the leg musculature of *Vireo* agree better with the condition in the cardinalines, emberizines, tanagers, wood warblers and icterines, than with that in the carduelines and ploceids. However, in a serological study, Stallcup (1961) found that *Vireo* is not particularly close to the tanagers (*Piranga*) or to the cardinalines (*Cardinalis*). In fact, *Vireo* was not close to any of the other oscines with which it was compared, including *Lanius*.

Bock (1960:471) considered the vireos to be "the most likely representatives of the ancestral nine-primaried stock" but, after listing the anatomical characters upon which he based this opinion, he concluded (1960:472) that "this evidence is not very conclusive and much more is needed to verify this hypothesis."

Bock (1962) found that the vireos exhibit only the beginnings of a second fossa in the head of the humerus. The shrikes have a single fossa and in the American nine-primaried groups the fossa is double.

Sibley (1970) reviewed the evidence and concluded that "there is general agreement . . . that the vireos are probably allied to the New World nine-primaried oscines, but not as closely as the members of the latter group are to one another." Sibley interpreted the electrophoretic patterns of the egg-white proteins as supporting this position.

Barlow and James (1975) studied the behavior and nesting of the Chestnut-sided Shrike-Vireo (*Vireolanius melitophrys*) and concluded that the shrike-vireos should be included in the Vireonidae.

Raikow (1978) compared the appendicular musculature of the New World nine-primaried oscines and the vireos. He (1978:41) concluded that the Vireonidae, including *Cyclarhis* and *Vireolanius*, are not closely related to the nine-primaried assemblage, but that their position "remains problematical because their affinities are still obscure."

Orenstein and Barlow (1981:36) concluded that the variations in jaw musculature which they observed in the Vireonidae "seemed related to differences in foraging technique . . . and of little use in determining intrageneric relationships." The jaw muscle variants were correlated with certain groupings within *Vireo* "but their chief importance . . . has been as indicators of the range and kind of morphological variation that has occurred in the Vireonidae as a whole" (1981:36). Orenstein and Barlow (1981) also recommended the inclusion of *Cyclarhis* and *Vireolanius* in the Vireonidae.

The reduction of the outer primary in the vireos is correlated with the extent of migration, as demonstrated by Averill (1925) and Hamilton (1958, 1962). In species with the longest migrations the outer primary is reduced the most, producing a longer, more pointed wing. Stegmann (1962) also found a correlation between the reduction of the outer primary and strong

flight. Conversely, sedentary species, especially those living in dense vegetation, tend to evolve rounded wings with long outer primaries.

## METHODS

To examine the taxonomic relationships between the genus *Vireo* and other passerines we have used the technique of DNA-DNA hybridization. Our procedures were based primarily upon those of Britten and Kohne (1968), Kohne (1970), and Britten et al. (1974). In our study of the ratite birds (Sibley and Ahlquist 1981a), we have described the technique in moderate detail, including a more extensive review of the relevant literature. The following is a synopsis of the methods used in the present study.

The genetic material, deoxyribonucleic acid (DNA), is a double-stranded molecule composed of linear sequences of four types of subunits (nucleotides) which differ in the chemical structures of their nitrogenous "bases," namely, adenine (A), thymine (T), guanine (G) and cytosine (C). In double-stranded DNA the bases occur as complementary pairs: an A in one strand pairs only with a T in the other strand, a G pairs only with a C. Genetic information is encoded in the sequences of the bases. The two strands of native DNA molecules will separate if heated in solution to ca. 100°C which ruptures ("melts") the hydrogen bonds between base pairs. Upon cooling, the double-stranded molecule re-forms because the complementary bases "recognize" one another and reassociate. If the temperature is maintained at or near 60°C base pairing will occur only between long homologous sequences of nucleotides. This is because only long sequences of complementary bases have sufficient bonding strength to maintain stable duplexes at that temperature, and only homologous sequences possess the necessary degree of complementarity. Thus, under appropriate conditions of temperature and salt concentration, the dissociated single strands of conspecific DNA will reassociate only with their homologous partners and the matching of complementary base pairs will be essentially perfect.

Similarly, if the single-stranded DNAs of two different species are combined under conditions favoring reassociation, "hybrid" double-stranded molecules will form between homologous sequences. These hybrid molecules will contain mismatched base pairs because of the nucleotide sequence differences (i.e., nucleotide substitutions) that have evolved since the two species diverged from their most recent common ancestor. The lower bonding strength of such hybrid molecules will cause them to dissociate at a temperature lower than that required to melt conspecific double-stranded DNA molecules. The reassociation of homologous sequences and the decreased thermal stability of imperfectly base-paired hybrid sequences form the basis of the DNA-DNA hybridization technique.

The extent of base-pair matching between the homologous nucleotide sequences of any two DNAs can be determined by measuring (1) the percentage of hybridization and (2) the thermal stability of the reassociated double-stranded molecules.

DNAs of the species in Table 1 were obtained from the nuclei of avian erythrocytes, purified according to the procedures of Marmur (1961) and Shields and Straus (1975), and "sheared" by sonication into fragments with an average length of ca. 500 nucleotides. Fragment size was determined by electrophoretic comparisons with DNA fragments of known size produced by the digestion of bacteriophage DNA with bacterial restriction endonucleases (Nathans and Smith 1975).

The single-stranded DNA fragments of the Red-eyed Vireo were allowed to reassociate to a $C_0t$ of 1000 at 50°C in 0.48 M sodium phosphate buffer. ($C_0t$ = the concentration of DNA in moles/liter × the duration of incubation in seconds [Kohne 1970:334].) This period of reassociation permitted most of the rapidly reassociating repeated sequences to form double-stranded molecules while the slowly reassociating single-copy sequences remained

single stranded. The latter were recovered by chromatography on a hydroxyapatite column. This process produced a single-copy DNA preparation consisting of one copy per genome of each original single-copy sequence and at least one copy per genome of each different repeated sequence. Such a single-copy preparation contains at least 98%, and probably 100%, of the "sequence complexity" of the genome, i.e., the total length of different DNA sequences (Britten 1971; R. J. Britten, pers. comm.). Kohne (1970:334–347) has discussed the reasons for using only single-copy DNA in studies designed to determine "the extent of nucleotide change since the divergence of two species."

The single-copy DNA sequences of the Red-eyed Vireo were labelled with radioactive iodine ($^{125}I$) according to the procedures of Commorford (1971) and Prensky (1976). DNA-DNA hybrids were formed from a mixture composed of one part (=250 ng) radioiodine-labelled single-copy DNA and 1000 parts (=250 $\mu$g) sheared, whole DNA. The hybrid combinations were heated to 100°C for 10 min to dissociate the double-stranded molecules into single strands, then incubated for 120 h (=$C_0t$ 16,000) at 60°C to permit the single strands to form double-stranded hybrid molecules.

The DNA-DNA hybrids were bound to hydroxyapatite columns immersed in a temperature-controlled water bath at 55°C and the temperature was then raised in 2.5°C increments from 55°–95°C. At each of the 17 temperatures the single-stranded DNA produced by the melting of double-stranded hybrids was eluted in 20 ml of 0.12 M sodium phosphate buffer.

The radioactivity in each eluted sample was counted in a Packard Model 5220 Auto-Gamma Scintillation Spectrometer, optimized for $^{125}I$. A teletype unit connected to the gamma counter printed out the data and punched a paper tape which is the entry to the computer program.

The computer program uses a non-linear regression least squares procedure to determine the best fit of the experimental data to one of four functions: (1) the Normal, (2) the dual-Normal, (3) the "skewed" Normal, or (4) a modified Fermi-Dirac. The $T_{50}H$ is the temperature above which less than 50% of the sequences will be hybridized, and below which more than 50% will be hybridized. This is, approximately, the median divergence point. The $T_{50}H$ is also the mode of a homologous hybrid and is equal to the mode of any hybrid if all single-copy sequences in the two species could form stable duplexes under the incubation conditions.

In each experimental set the labelled taxon is hybridized with itself (=homologous hybrid) and the differences in degrees Celsius between its $T_{50}H$ value and those of the heterologous hybrids are the delta $T_{50}H$ values. The $T_{50}H$ of Bonner et al. (1981) is the same as the $T_{50}R$ of Kohne (1970).

## RESULTS AND DISCUSSION

Table 1 contains the distance values (delta $T_{50}H$) for DNA-DNA hybrids between the Red-eyed Vireo and 35 other species of passerine birds. The delta $T_{50}H$ values are measurements between the Red-eyed Vireo and the other taxa, but not among the other taxa, because only the DNA of the Red-eyed Vireo was labelled with radioiodine. Two taxa that have the same delta $T_{50}H$ values are equidistant from the labelled taxon but they can be any distance from one another which is equal to, or less than, their common distance from the labelled species. Only a complete matrix of DNA distance values provides the basis for the reconstruction of a phylogeny but a one-dimensional data set, as in Table 1, can reveal the degrees of relationship between the labelled species and other taxa.

## Table 1

DNA-DNA Hybridization Values (delta $T_{50}H$) for Comparisons Between the Red-eyed Vireo and Other Species of Passerine Birds

| Species | Delta $T_{50}H$ | Group index[a] |
|---|---|---|
| Subfamily Vireoninae | | |
| 1. *Vireo olivaceus* (Red-eyed Vireo) | 0.0 | CV |
| 2. *V. altiloquus* (Black-whiskered Vireo) | 0.7 | CV |
| 3. *V. solitarius* (Solitary Vireo) | 2.5 | CV |
| 4. *V. solitarius* (Solitary Vireo) | 2.7 | CV |
| 5. *V. griseus* (White-eyed Vireo) | 2.9 | CV |
| 6. *Hylophilus ochraceiceps* (Tawny-crowned Greenlet) | 3.2 | CV |
| 7. *H. flavipes* (Scrub Greenlet) | 3.5 | CV |
| 8. *Cyclarhis gujanensis* (Rufous-browed Peppershrike) | 4.1 | CV |
| 9. *C. gujanensis* (Rufous-browed Peppershrike) | 4.2 | CV |
| Other members of the "corvine assemblage" | | |
| 10. *Coracina novaehollandiae* (Large Cuckoo-shrike) | 7.5 | CC |
| *11. *Grallina cyanoleuca* (Australian Magpie-lark) | 7.5 | CM |
| 12. *Dicrurus paradiseus* (Paradise Drongo) | 7.8 | CM |
| *13. *Monarcha guttula* (Spot-winged Monarch) | 7.8 | CM |
| 14. *Pica pica* (Black-billed Magpie) | 8.2 | CC |
| *15. *Corvus brachyrhynchos* (Common Crow) | 8.9 | CC |
| 16. *Aegithina tiphia* (Common Iora) | 9.2 | C? |
| *17. *Corvus brachyrhynchos* (Common Crow) | 9.4 | CC |
| *18. *Lanius collurio* (Red-backed Shrike) | 9.4 | CC |
| Members of other oscine families | | |
| 19. *Zonotrichia albicollis* (White-throated Sparrow) | 10.7 | FP |
| 20. *Geothlypis trichas* (Common Yellowthroat) | 10.8 | FP |
| *21. *Ploceus capensis* (Cape Weaver) | 11.0 | FP |
| *22. *Nectarinia jugularis* (Olive-backed Sunbird) | 11.1 | FP |
| *23. *Motacilla alba* (White Wagtail) | 11.2 | FP |
| 24. *Irena puella* (Fairy Bluebird) | 11.4 | ? |
| 25. *Lonchura bicolor* (Bronze Mannikin) | 11.4 | FP |
| *26. *Polioptila melanura* (Black-tailed Gnatcatcher) | 11.4 | ST |
| *27. *Passer domesticus* (House Sparrow) | 11.5 | FP |
| *28. *Trichastoma bicolor* (Ferruginous Jungle-Babbler) | 11.7 | ST |
| *29. *Sturnus vulgaris* (Common Starling) | 12.2 | MT |
| 30. *Meliphaga flaviventer* (Buff-breasted Honeyeater) | 12.4 | AWH |
| *31. *Mimus polyglottos* (Northern Mockingbird) | 12.5 | MT |
| *32. *Zosterops pallida* (Pale White-eye) | 12.8 | ST |
| *33. *Parus major* (Great Tit) | 13.2 | ST |
| *34. *Troglodytes aedon* (House Wren) | 13.2 | ST |
| *35. *Turdus migratorius* (American Robin) | 13.3 | MT |
| 36. *Ficedula dumetoria* (Orange-breasted Flycatcher) | 13.3 | MT |
| *37. *Sylvia atricapilla* (Blackcap) | 13.5 | ST |
| Suborder Tyranni | | |
| *38. *Elaenia flavogaster* (Yellow-bellied Elaenia) | 16.1 | SO |

[a] Group index abbreviations are: CV = Corvine-Vireonine; CC = Corvine-Corvine; CM = Corvine-Monarchine; FP = Fringillid-Ploceid; ST = Sylviine-Timaliine; MT = Muscicapine-Turdine; AWH = Australian Warbler-Honeyeater; SO = Suboscine; ? = uncertain.

* Indicates that the taxon has been used as the "labelled" species in another study.

From other studies (Sibley and Ahlquist 1980, 1981a–c, 1982b, c, in press a–c) we have found that (1) congeneric species commonly have delta $T_{50}H$ values up to ca. 3.0, (2) members of the same subfamily may differ by delta $T_{50}H$ values up to ca. 6.0, (3) members of the same family may differ by delta values up to ca. 9, and (4) species in different families of the same superfamily differ by delta $T_{50}H$ values between ca. 10 and 14. These estimates may vary by ±1.0 and delta $T_{50}H$ values have an average experimental error of ±0.5.

The delta $T_{50}H$ values in Table 1 indicate that *Vireo*, *Hylophilus* and *Cyclarhis* are closely related and may be included in the same subfamily. The delta $T_{50}H$ value for the Red-eyed Vireo × Black-whiskered Vireo hybrid (0.7) reflects the close relationship between these species. Mayr and Short (1970:72) include them in the same superspecies and note that *V. altiloquus* is "essentially the West Indian representative of *olivaceus* . . . ."

The taxa in Table 1 with delta $T_{50}H$ values from 7.5 (*Coracina*) to 9.4 (*Lanius*) are members of a large "corvine assemblage," the boundaries of which are not yet completely delineated. The group seems primarily to be Australasian and, in addition to the taxa represented in Table 1, it includes the Old World orioles (Oriolidae), the birds of paradise (Paradisaeidae), the Australo-Papuan bell-magpies and butcherbirds (Cracticidae) and the woodswallows (*Artamus*).

The taxa in Table 1 with delta $T_{50}H$ values from 10.7 (*Zonotrichia*) to 13.5 (*Sylvia*) are members of other families of the suborder Passeres. *Zonotrichia* and *Geothlypis* are representatives of the subfamily Emberizinae, family Fringillidae (Sibley 1970, Sibley and Ahlquist 1982b) and *Ploceus*, *Nectarinia*, *Motacilla*, *Lonchura*, and *Passer* are also members of the fringillid-ploceid assemblage (Sibley and Ahlquist 1980, 1981b, c). *Trichastoma* and *Sylvia* are members of the Sylviidae and *Parus* is related to them (Sibley and Ahlquist 1980, 1982c). *Sturnus*, *Mimus*, *Turdus* and *Ficedula* are members of a muscicapoid assemblage which includes the muscicapine flycatchers, erithacine thrushes, dippers (*Cinclus*), starlings, mockingbirds (*Mimus*) and thrashers (*Toxostoma*) and the turdine thrushes (Sibley and Ahlquist 1980, 1982c). The honeyeaters (e.g., *Meliphaga*) are members of an Australasian group that includes the acanthizine warblers (*Acanthiza*, etc.) and the fairy-wrens (*Malurus*, etc.) (Sibley and Ahlquist, in press b, c). The suboscine flycatcher, *Elaenia*, is a member of the family Tyrannidae.

The DNA hybridization data in the papers cited above provide a more extensive matrix of DNA distance values and have made it possible for us to define the principal clusters and the branching pattern of the taxa in Table 1. The results are diagrammed in Fig. 1.

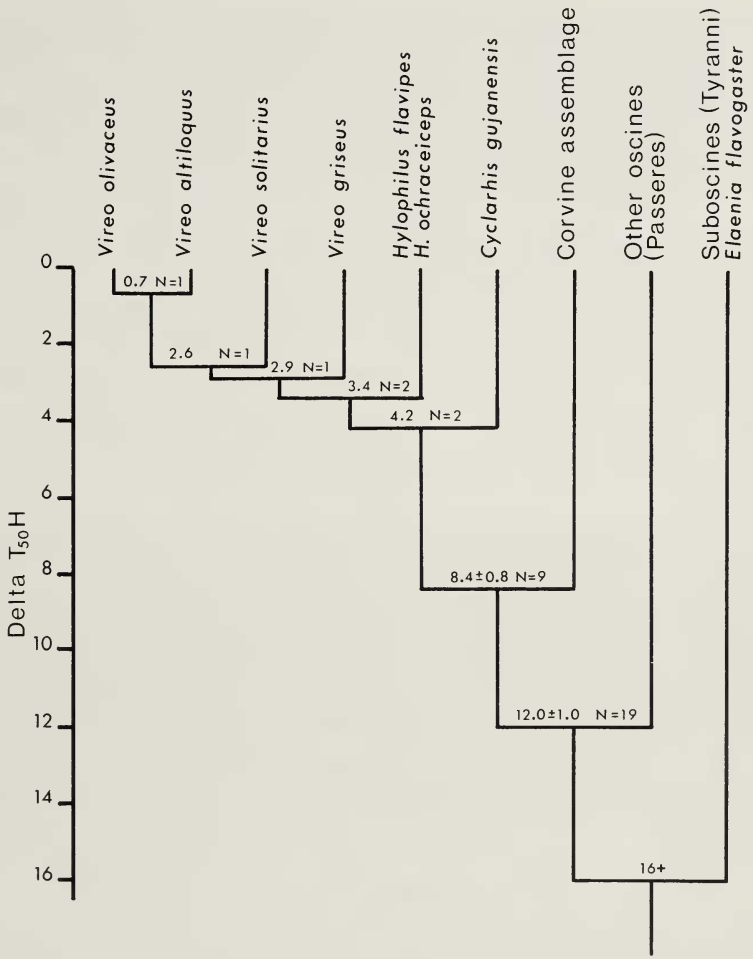


FIG. 1. Diagram based on the $T_{50}H$ values in Table 1. See text for explanation.

Each of the members of the vireonine cluster in Fig. 1 diverged from the lineage leading to *V. olivaceus* via different branch nodes; hence they form a nesting series of sister groups from *V. altiloquus* at delta $T_{50}H$ 0.7 to *Cylarhis* at 4.2. From the other studies cited above we know that the "corvine assemblage" includes the taxa in Table 1 with delta $T_{50}H$ values from 7.5–9.4. Since these are clustered within a range of 1.9 delta $T_{50}H$ units we may assume that this cluster is the sister group of the vireonines and we can therefore use the average delta $T_{50}H$ value (8.4 ± 0.8 SD) as the index to the divergence between these two clusters. Similarly, the

cluster of "other oscines" in Table 1 and Fig. 1 is the sister group of the combined vireonine and corvine clusters. The 19 genera in the cluster of "other oscines" represent 16 of Wetmore's (1960) families and have an average delta $T_{50}H$ of 12.0 ± 1.0 from *V. olivaceus*. Thus, although we may safely assume that these 16 families branched from one another over a span of at least several million years, they are all essentially the same delta $T_{50}H$ distance from the labelled species *V. olivaceus*. Furthermore, the corvine-vireonine lineage obviously branched from the "other oscines" lineage before these 16 families branched from one another.

We have found this same pattern of clustering and branching in all of our studies using DNA-DNA hybridization (cited above). This pattern indicates that all living taxa on one branch of a divergence node will be equidistant from all living taxa on the other branch of that node, i.e., all members of sister group A are equidistant from all members of sister group B, and vice versa. This is the "relative rate test" of Sarich and Wilson (1967).

We interpret this consistent pattern in the data as evidence that the average rate of DNA evolution (i.e., nucleotide substitution) is the same in all lineages of birds. This uniform average rate is apparently the statistical result of the large number of nucleotides in the avian haploid genome, viz., ca. $1.7 \times 10^9$. Each sequence evolves at its own rate and different sequences evolve at different rates at different times and in different species, but when averaged over the genome, and over time, the uniform average rate is the inevitable result because there are upper and lower bounds to the rates and their frequency distribution is narrow relative to the number of nucleotides.

The uniform average rate means that the DNA-DNA hybridization values are directly proportional to relative time and, therefore, may be used to reconstruct phylogenies. If they can be calibrated against geological or fossil dates, the DNA values can be correlated with absolute time. We have made some preliminary correlations (Sibley and Ahlquist 1981a), but the available datings are of uncertain accuracy and, so far, add little to the interpretation of the data. We urgently need accurate datings of the divergences between living lineages of birds.

We conclude that the typical vireos (*Vireo*), greenlets (*Hylophilus*), peppershrikes (*Cyclarhis*) and, presumably, shrike-vireos (*Vireolanius*) are members of the same subfamily, Vireoninae. It is not yet clear to which family the Vireoninae should be assigned but they are part of the corvine assemblage and are not closely related to the New World nine-primaried oscines. Additional support for this conclusion was obtained during a DNA-DNA hybridization study of the Hawaiian honeycreepers (Fringilli-

dae: Carduelinae: Drepanidini) in which a DNA-DNA hybrid between the radioiodine-labelled single-copy DNA of the Apapane (*Himatione sanguinea*) and the DNA of *V. olivaceus* had a delta $T_{50}H$ of 11.3 (Sibley and Ahlquist 1982a). Similarly, a DNA hybrid between the labelled DNA of the Yellow-breasted Chat (*Icteria virens:* Fringillidae: Emberizinae: Parulini) and the DNA of *V. olivaceus* had a delta mode of 10.4 (Sibley and Ahlquist 1982b).

Avise et al. (1980) also demonstrated the large genetic gap between the vireos and the wood warblers by their electrophoretic comparisons of 16 proteins among 12 genera of paruline warblers, a turdine thrush (*Catharus*) and the Red-eyed Vireo. They found that the thrush, although itself quite distant from the wood warblers, was closer to them than was the vireo. We have discussed this study in more detail elsewhere (Sibley and Ahlquist 1982b).

## SUMMARY

The relationships of the vireos (Vireoninae) have been debated for more than a century. The shrikes (Laniidae) and the New World nine-primaried oscines have been most frequently proposed as their closest relatives. Comparisons were made between the single-copy DNA sequences of the Red-eyed Vireo (*Vireo olivaceus*) and those of 34 other species representing other vireonines and the major taxa of passerine birds. *Vireo*, *Hylophilus* and *Cyclarhis* were found to be closely enough related to one another to be placed in the same subfamily. Their next nearest relatives are the members of the large and varied "corvine assemblage" which includes the cuckoo-shrikes, drongos, monarch flycatchers, crows, shrikes and several other taxa.

The vireos are not closely related to the New World nine-primaried oscines. The belief in this alliance, which has been accepted for the past 50 years, was based upon the incorrect interpretation of the reduction of the outer (10th) primary and other morphological characters.

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